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“Does Drought Influence the Relationship between Biodiversity and Ecosystem Functioning in Boreal Forests?”

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Author contribution:

DB, AG¹ and AG² designed the experimental study. DB and CG conducted the field work. TJ provided the productivity data. CG, DB, TJ, AG¹ and AG² analyzed the results. CG and DB wrote the first draft of this manuscript and all authors substantially contributed to revisions.

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32

33 Abstract

34 In mixed forests, interactions among species influence ecosystem functioning but
35 environmental conditions also play an important role in shaping relationships between
36 biodiversity and ecosystem functioning. In the context of climate change, the carbon and
37 water balance in pure vs. mixed forest stands may be differentially influenced by changing
38 soil water availability. To test this hypothesis, we compared the influence of biodiversity on
39 water use efficiency (WUE_s) in boreal forests between wet and dry years. We assessed the
40 carbon isotope composition ($\delta^{13}\text{C}$) of tree rings in *Betula pendula*, *Pinus sylvestris* and *Picea*
41 *abies* growing in pure vs. mixed stands. In addition, we tested whether differences in WUE_s
42 affected patterns of basal area increment (BAI_s). No biodiversity effect was found for stand
43 $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_s$) during the wet year. However, there was a significant increase in $\delta^{13}\text{C}_s$ between
44 the wet and the dry year and a significant effect of biodiversity on $\delta^{13}\text{C}_s$ in the dry year. The
45 increase in $\delta^{13}\text{C}_s$ in mixed stands was associated with both selection and complementarity
46 effects. Although BAI_s decreased significantly in the dry year, changes in $\delta^{13}\text{C}_s$ did not
47 translate into variations in BAI_s along the biodiversity gradient. Our results confirmed that the
48 physiological response of boreal forest ecosystems to changing soil water conditions is
49 influenced by species interactions and that during dry growing seasons, species interactions in
50 mixed stands can lead to lower soil moisture availability. This illustrates that biodiversity
51 effects can also be negative in mixed stands in the sense that soil resources can be more
52 intensively exhausted. Overall, our results confirm that in boreal forests, the biodiversity-
53 ecosystem functioning relationship depends on local environmental conditions.

54 **Key-words:** biodiversity, boreal forest, drought, $\delta^{13}\text{C}$, mixed forest, water use efficiency

55 INTRODUCTION

56 The biodiversity and ecosystem functioning (B-EF) relationship has received growing interest
57 from ecologists throughout the last two decades due to the rapid loss of diversity observed
58 during the last century (Symstad and others 2003). Several mechanisms have been put
59 forward to explain how community composition and richness can either negatively or
60 positively influence ecosystem functions. A negative influence on biodiversity can result from
61 direct competition for resources among species in a given ecosystem. Negative interactions
62 commonly arise when ecological niches overlap and/or when species share the same
63 functional characteristics and are thus functionally redundant in the ecosystem (Naeem 2008;
64 Vilà and Sardans 1999). In contrast, positive B-EF relationships are commonly attributed to
65 two other mechanisms: complementarity and selection. Complementary use of resources
66 among species refers both to ecological niche partitioning and facilitation (Loreau and Hector
67 2001) and implies species coexistence without major interspecific competition for resources.
68 The selection effect recognises that the probability of occurrence of high-performing species
69 is greater in highly diverse ecosystems (Loreau and Hector 2001). Studies investigating
70 complementarity and selection effects mainly focused on ecosystem productivity (e.g.
71 Bradford 2011; Fargione and others 2007; Isbell and others 2009; Morin and others 2011;
72 Zhang and others 2012), while only a few were interested in other ecosystem functional traits
73 (e.g. Forrester and others 2010; Kunert and others 2012; Meinen and others 2009).
74 In addition to competition, complementarity and selection effects, local environmental
75 conditions also play an important role in shaping B-EF relationships (Belote and others 2011;
76 Hooper and Dukes, 2004). Under the assumptions of the “stress-gradient” hypothesis, which
77 predicts that the net outcome of biotic interactions (competition and facilitation) shifts from
78 negative to positive along gradients of limiting physical conditions (Bertness and Callaway
79 1994), positive biodiversity effects are expected to be more common in severely resource-

limited conditions while negative effects should prevail in richer and milder environments. This general conceptual model has recently been refined (Maestre and others 2009) and is widely supported in the literature (Herbert and others 2004; Jucker and Coomes 2012; Steudel and others 2012; He and others 2013; Wang and others 2013). In the context of climatic change, most regions around the world are expected to encounter more extreme environmental conditions (IPCC, 2007). The “stress-gradient” hypothesis is therefore of great interest since B-EF relationships are likely to change in the future. Whether or not more diverse ecosystems might be better adapted and/or more resilient to these changes is an important issue to investigate.

In the boreal climate zone, simulations predict a general shift from short, cool summers towards longer, warmer summers (IPCC 2007; Jylhä and others 2010). Thus, boreal forest ecosystems are expected to encounter more frequent and intense reduced soil water availability in summer. Contrasted responses of carbon and water fluxes in forest ecosystems to warmer and drier climate have already been observed (reviewed in Boisvenue and Running 2006). Few studies so far were conducted on the response of boreal forests to these conditions. Nevertheless, Dulamsuren and others (2010) showed that increasing summer temperatures accompanied by decreasing precipitation lead to reduced productivity in taiga forests in Mongolia.

Plants adapt to reduced soil water conditions through numerous physiological and/or morphological processes (review in Kozłowski and Pallardy 2002). At the leaf level, under drought conditions, they must manage the trade-off between optimum carbon gain for growth and loss of water through transpiration (Farquhar and others 1982) which usually results in an increase in intrinsic water use efficiency (WUE_{int}), defined as the ratio between CO_2 assimilation during photosynthesis and stomatal conductance for water vapour (e.g. Zhang and Marshall 1994). At ecosystem level, carbon and water fluxes are influenced by species-

specific functional responses to environmental conditions and by intra- and inter-specific interactions both below- and above-ground. Both positive and negative interactions among species may occur in mixed stands and this may lead to differing spatial and temporal resource availability and physiological and morphological adaptations within species. These complex interactions mean that general predictions on stand-level water use efficiency (WUE_s) under dry conditions cannot solely be based on individual species responses to these conditions.

In this context, little information is available concerning the relationship between tree biodiversity and the regulation of carbon and water fluxes in boreal forest ecosystems. Gamfeldt and others (2013) found positive relationships between tree species richness and multiple ecosystem services (tree biomass, soil carbon storage, berry production and game production potential) in production forests in Sweden and explained the observed positive B-EF relationship by facilitation processes among tree species. In contrast, Grossiord and others (2013a) found no complementary effect for biomass production and WUE_s in a young boreal plantation, though they did find a weak selection effect. These studies, however, did not compare the B-EF relationship in contrasted environmental conditions.

In this study, we tested the following assumptions: i) in boreal forest ecosystems, the stand-level carbon and water balance under non-limiting soil water conditions (wet year) should not necessarily depend on biodiversity effects, and ii) under limiting soil water conditions (dry year), species identity, species combinations and richness levels should influence ecosystem functioning. We analyzed the influence of species richness on time-integrated WUE_s estimated from the carbon isotope composition ($\delta^{13}C$) (Farquhar and others 1982) of ring whole wood measured in tree rings from two years with contrasting soil water conditions (wet year in 2004 and dry year in 2006). Samples were taken from 26 mature stands in Eastern Finland, which were either pure or mixed with varying percentages of *Betula pendula*, *Pinus*

130 *sylvestris* and *Picea abies*. We also tested whether changes in WUE_s would influence stand
131 basal area increment (BAI_s).

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133

MATERIALS AND METHODS

Site description

The study was conducted in August 2012 in 26 stands of boreal forest that are distributed over a 2000 km² area around Joensuu, Finland (62.60°N, 29.76°E). The stands were 30 m x 30 m and included pure, 2-species mixture, or 3-species mixture of Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*) and Silver birch (*Betula pendula*). Four replicates of each combination were selected except for pure birch stands that were sampled only twice. Mean tree age within the stands was 33.9 ± 5.8 years. Tree species characteristics are given in Table 1.

The stands were selected according to the following specifications: no change in management had occurred in the last 5 years; confounding factors like altitude, topography or soil type were kept to a minimum between selected stands; in a given forest patch, the stands were more than 500 m apart to avoid spatial autocorrelation; stands were mostly even-aged and single-layered. More detailed information on the selection procedure of the study stands can be found at <http://www.fundiveurope.eu>. The selected stands were situated between 80 and 200 m a.s.l. on Podzols soils (FAO classification) above mica schist bedrock. Mean annual rainfall in the region is around 700 mm and mean annual temperature is 2.1°C.

Selection of the target years

The daily water balance model “BILJOU” (Granier and others 1999) was implemented in order to quantify soil water availability at a daily time-scale in the study region and then to select the driest and the wettest year over the past fifteen years. We used above-canopy measurements of daily meteorological conditions (rainfall, global radiation, air temperature and humidity, wind speed) from the Hyytiälä forestry field station (61.84°N, 24.28°E, 153 m

a.s.l.) to run the model. Although this meteorological station is situated 300 km south-west of Joensuu, it is the nearest source from which data could be obtained to estimate daily relative extractable soil water (REW) over the 1997-2011 period (Fig. 1). The model was initialized assuming that soil was at field capacity on 1st January. We used a leaf area index of 5.0 m² m⁻² and a soil holding capacity of 125 mm as input data for model simulations. We discarded the years 1998 and 2010 because of too many missing data. Soil water deficit was assumed to occur when the REW dropped below 0.4, a threshold value that has been shown to induce stomatal closure and interrupt radial growth in forest trees (Granier and others 1999). For each remaining year from 1997 to 2011, we characterized annual water stress using the starting day and the duration of the period when REW dropped below the threshold limit coupled with a water stress index which cumulates the difference between simulated REW and the 0.4 threshold (Granier and others 1999). These calculations revealed that in 2006 water stress was the most severe and in 2004 it was the least severe (Fig. 1). Drought started on June 16 in 2006 while there was no drought in 2004. Total precipitation during the growing season (May to September) was 157 and 303 mm in 2006 and 2004, respectively.

Wood carbon isotope composition

Six trees per species in each pure stand and three trees per species in mixed stands were randomly selected among the ten trees with the largest diameter at breast height. For each selected tree we extracted one 5-mm-diameter wood core at breast height (1.3 m) from the south side of the trunk. The fresh samples were shipped to INRA Nancy and oven-dried at 50°C for 72 hours to reach constant weight before being filed with a scalpel for tree-ring dating. For both target years, we carefully extracted the late wood with a scalpel. The wood fragments from a given species in a given stand were then pooled and ground into a fine powder. We combined the wood fragments from individuals of the same species in the same

stand to obtain enough material for carbon isotope analyses, to ensure adequate sample homogeneity, and to reduce experimentation costs. Although we sampled only a few trees per species in each stand, the level of replication was adequate given the imprint of environmental signals on tree-ring carbon isotope composition (e.g. Peñuelas and other 2008). From 0.4 to 1.5 mg of the ground wood samples were weighed on a high-precision scale (MX5, Mettler Toledo, Viroflay, FR) and placed into tin capsules (Elemental Microanalysis Limited, Devon, UK) for $\delta^{13}\text{C}$ analyses. Samples were analyzed with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility of UC Davis, CA, USA. All isotopic measurements are reported in the standard delta notation (δ , ‰):

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad [1]$$

where R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ ratios of the samples and the Vienna Pee Dee Belemnite (VPDB) standard.

Wood basal area increment

To quantify productivity, we randomly selected five trees per species from each pure stand, and three trees per species in mixed stands, among the twelve (pure stands) or eight (mixed stands) trees with the largest diameter at breast height. For each tree, one 5-mm-diameter wood core was extracted at breast height from the south side of the trunk. These cores were different from the ones taken for $\delta^{13}\text{C}$ analysis. Once air dried, the core samples were mounted on wooden boards and then sanded with paper of progressively finer grit sizes. A high resolution (2400 dpi) flatbed scanner was then used to image the cores, following which diameter growth measurements and crossdating were performed with the CDendro software suite (Cybis Elektronik & Data, Saltsjöbaden, Sweden). For each of the studied years, diameter growth measurements of the individual trees were converted to basal area increment,

then standardized basal area increment (BAI_i , $\text{cm}^2 \text{ cm}^{-2} \text{ yr}^{-1}$) was obtained by dividing basal area increment by the basal area of each tree.

Stand-level carbon isotope composition and basal area increment

Based on species-specific $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_i$) for each stand, we calculated stand-level carbon isotope composition ($\delta^{13}\text{C}_s$). The contribution of a given species to $\delta^{13}\text{C}_s$ depends on its species-specific contribution to stand CO_2 assimilation rates (Lloyd and Farquhar 1994). Consequently, when $\delta^{13}\text{C}_i$ values are scaled up from species level to stand level, $\delta^{13}\text{C}_i$ values should be weighted by species assimilation rates. However, since species-specific CO_2 assimilation rates were not available in this study, we used species total basal area (BA_i , cm^2) in each stand as a proxy, following Bonal and others (2000). $\delta^{13}\text{C}_s$ was thus calculated as:

$$\delta^{13}\text{C}_s = \frac{\sum_{i=1}^N BA_i \delta^{13}\text{C}_i}{\sum_{i=1}^N BA_i} \quad [2]$$

where N is the number of species in the mixture.

$\delta^{13}\text{C}$ in C_3 plants constitutes a record of the intercellular / atmospheric CO_2 concentration ratio during the period in which the carbon was fixed and is strongly positively correlated to leaf intrinsic water use efficiency (WUE_{int}) (Farquhar and others 1982) as shown in the following equation:

$$\delta^{13}\text{C}_s = \frac{a}{b} \ln \left(\frac{C_a}{C_i} \right) \quad [3]$$

where C_a is the CO_2 concentration in the atmosphere, δ_a and $\delta^{13}\text{C}$ are respectively the carbon isotope compositions of the atmosphere and of the sample, a represents the fractionation occurring due to diffusion in air (4.4 ‰), and b is the fractionation caused by carboxylation (27 ‰). We thus used Equation 3 to calculate stand-level water use efficiency (WUE_s) based on $\delta^{13}\text{C}_s$.

To estimate stand basal area increment (BAI_s , $\text{cm}^2 \text{cm}^{-2} \text{yr}^{-1}$) of the two studied years (2004 and 2006) and of the year following the driest year (2007), we considered the relative importance of each species in terms of contribution to total basal area. Thus, BAI_s was calculated as:

$$.$$
 [4]

Data analysis

All analyses were performed using the statistical software R 2.14.1 (R Development Core Team, 2011). We tested three effects that could impact $\delta^{13}\text{C}_i$, $\delta^{13}\text{C}_s$, BAI_i and BAI_s . Firstly, tree biodiversity, which we characterised for each stand with the Shannon biodiversity index. This index expresses the uncertainty of predicting the proportion of stand basal area a given tree species represents in binary digits (ranging from zero for pure stands to the logarithm in base two of the total basal area of the species in the stand). Secondly, competition intensity in each stand, which we expressed as the total basal area of the stand. Thirdly, year. We applied a series of mixed-effect models to analyze the impact of the three effects and their interactions on $\delta^{13}\text{C}_i$, $\delta^{13}\text{C}_s$, BAI_i and BAI_s with “stand” as a random effect. To test for any biodiversity effect during the recovery phase to drought (year 2007), we applied the same mixed-effect model on BAI_s . Contrasts were used to test for relevant differences between fixed effects when the overall model was significant. We calculated the difference in $\delta^{13}\text{C}_s$ and BAI_s between the dry and wet year ($\Delta\delta^{13}\text{C}_s$ and ΔBAI_s , respectively) and used Spearman regressions to test the relationship between the Shannon biodiversity index and $\Delta\delta^{13}\text{C}_s$ or ΔBAI_s .

When a significant effect of biodiversity on $\delta^{13}\text{C}_s$ or BAI_s was observed, we evaluated the net biodiversity effect, the complementarity effect, and the selection effect on $\delta^{13}\text{C}_s$ or BAI_s with

254 the method developed by Grossiord and others (2013b). We used one-way ANOVA and post-
255 hoc Tukey tests to check for differences in these biodiversity effects among species richness
256 levels. We used t-tests to check all three biodiversity effects for differences from 0 for each
257 mixture level and for each mixture type (identity of the mixture).

RESULTS

Wood carbon isotope composition

We observed considerable variability in $\delta^{13}\text{C}_i$ across species, species mixtures and years, ranging from -28.7 ‰ for birch in a 2-species mixture with spruce to -26.2 ‰ for spruce in a 2-species mixture with pine during the wet year, and from -27.4 ‰ for birch in a mixture with spruce to -24.2 ‰ for pine in a 3-species mixture during the dry year. Mixed-effect models revealed a significant species effect ($P < 0.001$), year effect ($P < 0.001$) and an interaction between Shannon index and year ($P = 0.019$), but no effect of the Shannon index alone, the interactions of the Shannon index and the species, or the interactions of the year and the species ($P > 0.050$). The significant effect of the interaction between Shannon index and year was only observed for pine and spruce ($P = 0.012$ and $P = 0.020$, respectively), indicating for these species a differential response of $\delta^{13}\text{C}_i$ to the mixture in the two years.

For each species, whatever the richness level, we found significantly higher values of $\delta^{13}\text{C}_i$ during the dry year than during the wet year ($P = 0.003$ for birch, $P = 0.017$ for pine and $P = 0.037$ for spruce). For the wet year, we did not observe any effect of the Shannon index or of competition intensity on $\delta^{13}\text{C}_i$ for any species ($P > 0.050$). In contrast, during the dry year, pine and spruce showed a significant positive Shannon index effect ($P = 0.013$ and $P = 0.027$, respectively) and a competition intensity effect ($P < 0.001$ and $P = 0.010$, respectively), whereas birch did not display any significant patterns ($P > 0.050$).

At plot level, $\delta^{13}\text{C}_s$ values ranged from -28.1 ‰ for a pine-spruce mixture to -26.6 ‰ for a pure stand of spruce during the wet year and from -26.9 ‰ in a pure stand of pine to -24.6 ‰ for a 3-species mixture during the dry year (Fig. 2). We observed a significant effect of year on $\delta^{13}\text{C}_s$ ($P < 0.001$), with higher values of $\delta^{13}\text{C}_s$ during the dry year, as well as a significant interaction between Shannon index and year ($P = 0.012$). Specifically, while there was no

significant relationship between the Shannon index or the competition index and $\delta^{13}\text{C}_s$ during the wet year ($P > 0.050$), a highly significant and positive Shannon index effect was apparent ($P = 0.008$) during the dry year, along with a marginal influence of competition intensity ($P = 0.067$). Differences in $\delta^{13}\text{C}_s$ between the dry and the wet year ($\Delta\delta^{13}\text{C}_s$) for each stand were positively correlated with the Shannon index ($P = 0.038$).

Wood basal area increment

The basal area increment (BAI_i) of individual species ranged from $0.3 \times 10^{-3} \text{ cm}^2 \text{ cm}^{-2} \text{ yr}^{-1}$ for pine mixed with birch to $52.9 \times 10^{-3} \text{ cm}^2 \text{ cm}^{-2} \text{ yr}^{-1}$ for spruce mixed with pine during the wet year, and from $0.7 \times 10^{-3} \text{ cm}^2 \text{ cm}^{-2} \text{ yr}^{-1}$ for pine mixed with birch to $41.7 \times 10^{-3} \text{ cm}^2 \text{ cm}^{-2} \text{ yr}^{-1}$ for birch mixed with spruce during the dry year. Mixed-effect models revealed a significant species effect ($P < 0.001$), year effect ($P < 0.001$) and an interaction between the species and the year ($P < 0.001$), but no effect of the Shannon index alone, the interactions between the Shannon index and species, or the interactions between the Shannon index and the year ($P > 0.050$). For spruce, we found significantly lower values of BAI_i during the dry year than during the wet year ($P < 0.001$), while for birch and pine, no significant changes in BAI_i were found. For the wet year, we did not observe any effect of the Shannon index or competition intensity on BAI_i for any species ($P > 0.05$). For the dry year, pine showed a significant positive Shannon index effect ($P = 0.035$) and no competition intensity effect ($P = 0.813$). Birch and spruce did not display any significant Shannon or competition effects during the dry year ($P > 0.050$).

BAI_s values ranged from $14.9 \times 10^{-3} \text{ cm}^2 \text{ cm}^{-2} \text{ yr}^{-1}$ in a birch-pine mixture to $61.3 \times 10^{-3} \text{ cm}^2 \text{ cm}^{-2} \text{ yr}^{-1}$ in a pure stand of spruce for the wet year and from $16.9 \times 10^{-3} \text{ cm}^2 \text{ cm}^{-2} \text{ yr}^{-1}$ in a birch-pine mixture to $59.8 \times 10^{-3} \text{ cm}^2 \text{ cm}^{-2} \text{ yr}^{-1}$ in a birch-spruce mixture for the dry year (Fig. 2). In 2007,

the year following the driest year, BAI_s values ranged from $16.8 \times 10^{-3} \text{ cm}^2 \text{ cm}^{-2} \text{ yr}^{-1}$ in a pure pine stand to $59.4 \times 10^{-3} \text{ cm}^2 \text{ cm}^{-2} \text{ yr}^{-1}$ in a pine-spruce mixture (Fig. 2). The interaction between the Shannon index and the year was not significant ($P = 0.976$). However, we observed a significant effect of the year on BAI_s ($P = 0.001$), with higher values during the wet year in comparison to the dry and the recovery years (Fig. 2). We did not detect any significant effect of the Shannon index or the competition index on BAI_s in either year ($P > 0.050$). We found that differences in BAI_s between the dry and the wet year (ΔBAI_s) were not correlated with the Shannon index ($P = 0.143$).

Biodiversity effects on stand carbon isotope composition

Overall, there was no significant change in either net, complementarity or selection effect on $\delta^{13}\text{C}_s$ with increasing species richness during the dry year ($P > 0.050$) (Fig. 3). However, when each mixture level was considered independently, complementarity, selection and net effects were significantly higher than zero in the 2-species mixtures ($P < 0.050$), and mainly positive - although not significantly different from 0 - in the 3-species mixtures ($P > 0.050$). When we tested each different species mixture for significance of the biodiversity effects, we found that none of the three effects were significantly different from zero ($P > 0.050$) in the pine-spruce mixtures. However, for birch-pine and birch-spruce mixtures, net and complementarity effects were significantly higher than zero ($P < 0.050$), while no selection effects emerged ($P > 0.050$).

DISCUSSION

1. Climate effect

Our stable isotope analysis revealed a strong physiological response in boreal trees to changing soil water conditions and helped clarify the mechanisms responsible for observed changes both at the leaf and stand level. The increase in both individual species' $\delta^{13}\text{C}_i$ and stand level $\delta^{13}\text{C}_s$ from wet to dry years (Fig. 2) reflects an active regulation of carbon and water fluxes in this boreal ecosystem and the strong sensitivity of the three species to limiting soil water conditions. Assuming that the difference in δ_a between the two selected years was negligible, the magnitude of the increase in $\delta^{13}\text{C}_s$ reflected a mean increase in WUE_s of $17.0 \pm 1.8 \mu\text{mol mol}^{-1}$ (i.e. $+19.2 \pm 1.5 \%$). This increase is consistent with the extensive body of literature documenting the functional response of boreal trees to drought conditions (e.g. Vaganov and others 2009; Zhang and Marshall 1994; Saurer and others 2004). This pattern indicates that in the context of climate change, when more frequent periods of water shortage in north-European regions can be expected (IPCC 2007), the three locally-adapted species studied here will be more efficient in using available water resources for carbon acquisition. However, carbon sequestration and storage rates in wood may well decline; indeed, we found that the increase in stand $\delta^{13}\text{C}_s$ during the dry year was associated with a decrease in BAI_s . This is unsurprising as numerous studies conducted in boreal forests have shown lower productivity under water limiting conditions (e.g. Kljun and others 2007).

2. Biodiversity effects under wet conditions

Our results confirmed our first hypothesis that under non-limiting soil water conditions there would be little or no effect of biodiversity on $\delta^{13}\text{C}_s$ and BAI_s (Fig. 2). Interactions among species under non-limiting soil water conditions did not lead to any clear B-EF relationships in our boreal study forests, which is consistent with the expectations of the “stress-gradient”

hypothesis (Bertness and Callaway 1994). This result is also consistent with a recent study we conducted over a wet summer on the influence of biodiversity on species- and stand-level carbon and water balance in a young boreal plantation in Finland (Grossiord and others 2013a). Therein, we argued that in addition to non-limiting soil water conditions, the young ontogenic stage of the plantation likely caused the absence of any biodiversity effect. However, the present study does not support this assumption. Similar observations made in other ecosystem types also concluded on the absence and/or the decrease of biodiversity effects under non-limiting environmental conditions (e.g. Hughes and Stachowicz 2004; Steudel and others 2012).

3. Contrasting biodiversity effects under wet and dry conditions

The major result of this study was the positive relationship between biodiversity and the difference in $\delta^{13}\text{C}_s$ between dry and wet years ($\Delta\delta^{13}\text{C}_s$) (Fig. 2). Drier conditions globally enhanced $\delta^{13}\text{C}_s$, and thus WUE_s , but this influence depended on species interactions. Even though the relationship was rather dispersed, stands with greater diversity showed the greatest increase in $\delta^{13}\text{C}_s$, pointing to a greater compromise between carbon accumulation and water consumption under limiting soil water conditions. However, those changes in ecosystem-scale physiological acclimatization to drought failed to minimize the decrease in BAI_s during the dry year and did not influence the productivity of these plots the following year (Fig. 2). The literature actually mentions this apparent inconsistency (e.g. Vaganov and others 2009). The compromise between carbon acquisition and water loss at leaf or plant level usually has a moderate effect on how much assimilated carbon is invested in secondary growth (Flanagan and Johnsen 1995). Carbon allocation to above- or below-ground components in response to soil water conditions strongly varies among species (e.g. Axelsson and Axelsson 1986) and could partially explain the absence of enhanced BAI_s in our study.

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377 4. Origin of the biodiversity effects

378 Partitioning the influence of biodiversity on $\delta^{13}\text{C}_s$ into selection and complementarity effects
379 revealed that during the dry year the proportionally greater increase in WUE_s observed in the
380 2-species mixtures was a result of both mechanisms (Fig. 3). Selection effects refer to a higher
381 performance of one or several species in mixtures on the cost of others. Therefore, the pattern
382 of increasing $\delta^{13}\text{C}_s$ we observed was most likely driven by the two conifers rather than by
383 birch, since both conifers showed higher $\delta^{13}\text{C}_i$ in 2-species mixtures while birch did not (Table
384 1). Higher $\delta^{13}\text{C}_s$ values in the 2-species mixtures with birch were also partially explained by a
385 complementarity effect, probably as a result of different rooting strategies among the species.
386 Birch is known to have deeper rooting systems that explore larger volumes than those of pine
387 and spruce, thus enabling birch to access both shallow and deep soil layers (Kalliokoski and
388 others 2008). The fact that birch $\delta^{13}\text{C}_i$ values did not respond to increasing diversity during the
389 dry year supports this assumption and suggests that the effects of intra- and inter-specific
390 interactions for water acquisition for this species are similar. In contrast, the complementarity
391 effect for $\delta^{13}\text{C}_s$ was not significant in stands where the two conifers cohabited. *Pinus*
392 *sylvestris* and *Picea abies* are known to present similar rooting system development when
393 cohabiting in mixed stands (Kalliokoski and others 2008). It therefore appears that the two
394 conifers were in part functionally redundant in terms of carbon and water acquisition and use
395 in these mixed stands.

396 Although in the 3-species mixtures none of the three biodiversity effects were significantly
397 different from zero, overall they were generally consistent with results for the 2-species mixed
398 stands (Fig. 3). Numerous B-EF studies have revealed such saturating or even hump-shaped
399 relationships (e.g. Gamfeld and others 2013; Vilà and others 2013). One explanation for this
400 type of pattern is increasing functional redundancy at higher levels of species richness (as

observed for the two conifers here), which leads to faster and more complete exploitation of available resources in stands with greater richness.

5. Other potential origins of the biodiversity effects

Variations in $\delta^{13}\text{C}$ in tree rings reflect physiological processes of acclimatization to environmental conditions and physical archives of short- or long-term changes in soil moisture and/or air humidity in forests (Saurer and others 2004; Andreu and others 2008; Mölder and others 2011). Therefore, the significantly higher $\delta^{13}\text{C}_s$ values we observed along the diversity gradient in the dry year could be viewed not only as a consequence of species interactions, but also as a result of lower soil water availability in mixed stands as compared to pure ones. High soil moisture exhaustion in mixed stands during dry years was already reported in other hydrological investigations in mixed species forests (Gebauer and others 2012). Such a feedback effect could arise because of the particularly high competitiveness of birch for water resources and its high water consumption (Kalliokoski and others 2008). This would lead to a higher drought exposure for the conifers in the mixed stands and thus reduced transpiration through stomatal regulation. This increased drought exposure may have been also the cause for the absence of any increase in BAIs in the mixed stands. Nevertheless, differences in canopy structure inducing different atmospheric humidity and light conditions between pure and mixed stands (Kelty 2006) as well as below-ground competition among these species for nutrients (nitrogen, phosphorus...) may also have contributed to the mixture effects we detected; however, this is beyond the scope of the present study.

424 CONCLUSION

425 In our boreal study region, we demonstrated a clear interaction between the
426 biodiversity/ecosystem carbon and water flux relationship and environmental conditions,
427 consistent with the “stress gradient” hypothesis. The increase in $\delta^{13}\text{C}_s$, and thus in ecosystem-
428 level water use efficiency, with drier soil conditions was much higher in mixed stands than in
429 pure ones, but this did not prevent a decrease in above-ground growth rates with drought. At
430 the species level, functional similarities between the two conifers lead to a redundant
431 contribution of these two species to ecosystem-level carbon and water balance. In contrast, the
432 presence of birch in mixed stands strongly impacted the water use efficiency of the conifers
433 under limiting water conditions. Our results suggest that species interactions in mixed stands
434 during dry growing seasons can lead to lower soil moisture availability as compared to pure
435 ones. This illustrates that biodiversity effects can also be negative in mixed stands in the sense
436 that soil resources can be more intensively exhausted. Our study highlights the need to take
437 into account not only species growth characteristics but also species functional traits when
438 managing boreal mixed forest in order to bolster mixed-forests in a context of climate change.

439

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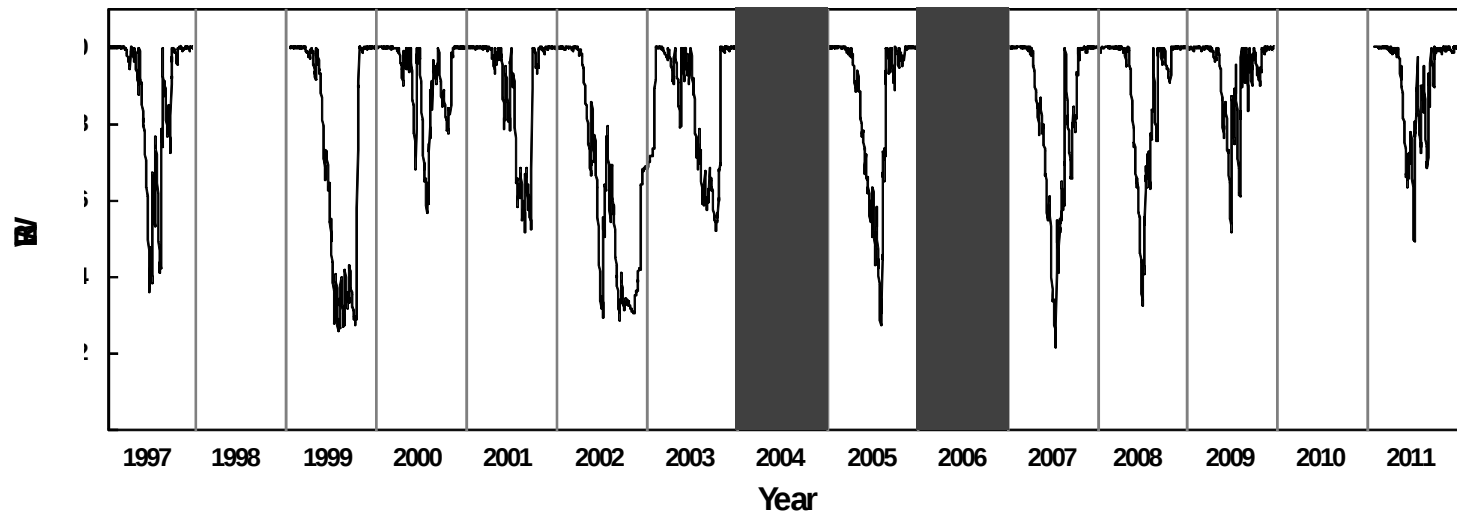
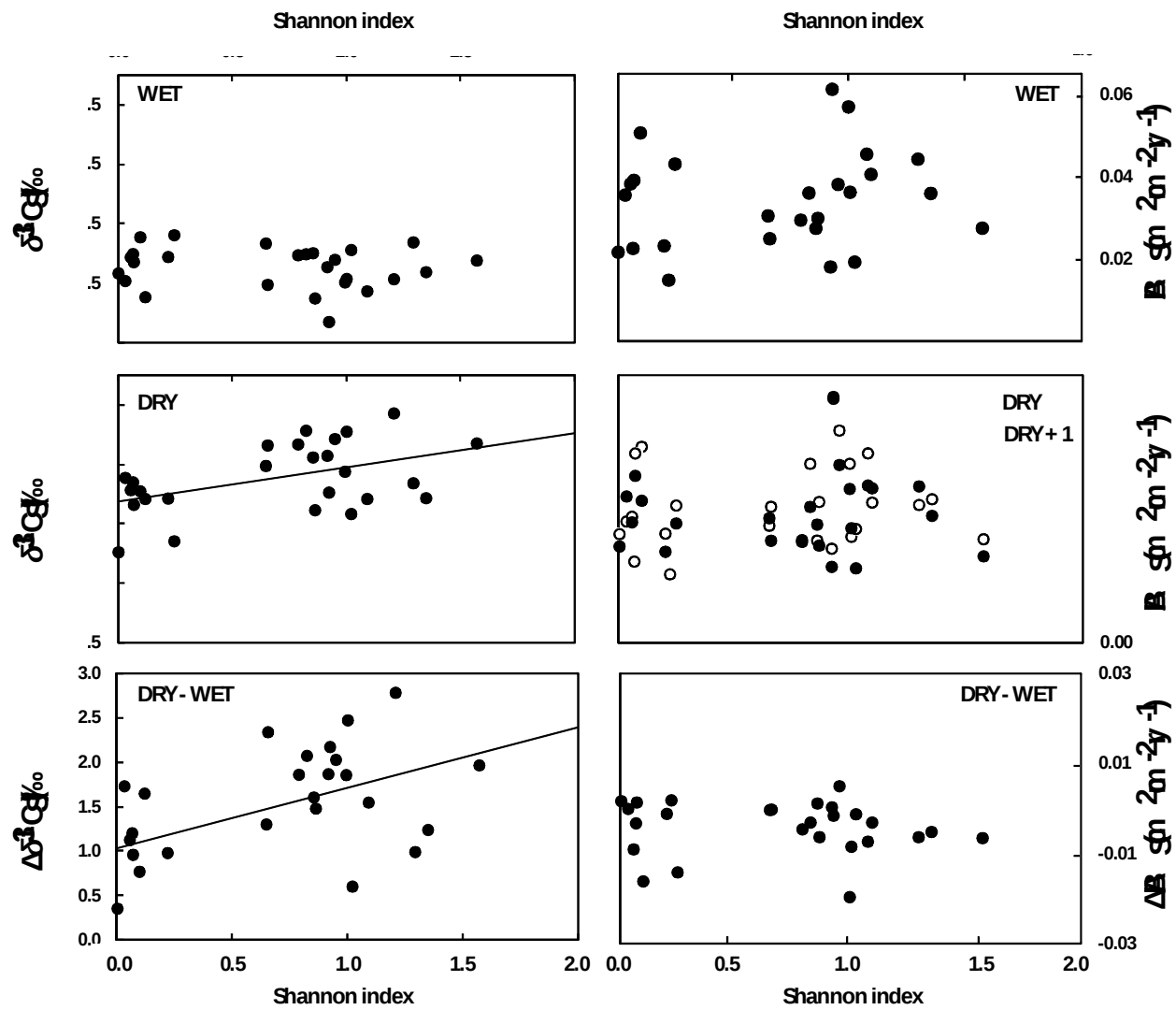


Figure 2

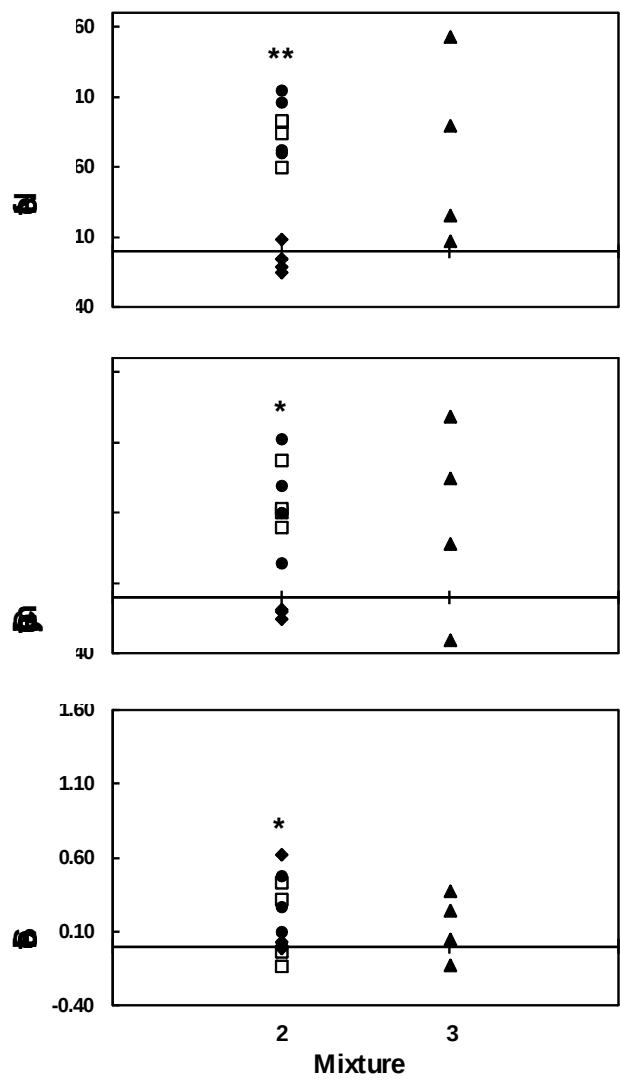


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Figure 3



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Figure legends:

Figure 1: Time-course of relative extractable water (REW) for the years 1997 to 2012 simulated with the “BILJOU” model. The two selected years are highlighted in grey (wet for 2004 and dry for 2006).

Figure 2: Stand carbon isotope composition ($\delta^{13}\text{C}_s$, ‰), stand size-standardized basal area increment (BAI_s , $\text{cm}^2 \text{ cm}^{-2} \text{ yr}^{-1}$) for the wet, the dry and the recovery year (DRY+1, white circles), and the differences in $\delta^{13}\text{C}_s$ and BAI_s between the wet and the dry year ($\Delta\delta^{13}\text{C}_s$ and ΔBAI_s , respectively) as a function of the Shannon biodiversity index. The black lines represent the fitted regression model when the model was significant ($R^2 = 0.22$ and 0.23 for $\delta^{13}\text{C}_s$ and $\Delta\delta^{13}\text{C}_s$, respectively).

Figure 3: Net, complementarity, and selection effects on stand carbon isotope composition ($\delta^{13}\text{C}_s$, ‰) for the dry year as a function of species richness. Black circles indicate birch and pine mixtures, white squares indicate birch and spruce mixtures, black diamonds indicate pine and spruce mixtures, and black triangles indicate 3-species mixtures. Asterisks denote significant differences from zero in the biodiversity effects for each species richness level (t-test, * $P < 0.05$; ** $P < 0.01$).

Table 1: Overall mean species diameter at breast height (cm) and mean height (m) with standard errors for *Betula pendula*, *Pinus sylvestris* and *Picea abies*.

Species	Mean diameter at breast height (cm)	Mean height (m)
<i>Betula pendula</i>	16.4 ± 0.2	17.9 ± 0.2
<i>Pinus sylvestris</i>	19.2 ± 0.1	17.6 ± 0.1
<i>Picea abies</i>	17.7 ± 0.2	16.1 ± 0.1

Table 2: Mean species-specific Shannon biodiversity index, wood carbon isotope composition ($\delta^{13}\text{C}_i$, ‰), and size-standardized basal area increment (BAI_i, cm² cm⁻² yr⁻¹) for the dry and wet years, with standard errors for *Betula pendula*, *Pinus sylvestris* and *Picea abies*, and for each mixture level.

Species	Mixture	Shannon	$\delta^{13}\text{C}_i$ Wet (‰)	$\delta^{13}\text{C}_i$ Dry (‰)	BAI _i Wet (cm ² cm ⁻² yr ⁻¹)	BAI _i Dry (cm ² cm ⁻² yr ⁻¹)
<i>Betula pendula</i>	1	0.2 ± 0.1	-27.1 ± 0.3	-25.6 ± 0.1	17.1×10 ⁻³ ± 0.8×10 ⁻³	18.2×10 ⁻³ ± 0.9×10 ⁻³
	2	0.9 ± 0.1	-27.8 ± 0.2	-25.8 ± 0.3	19.2×10 ⁻³ ± 0.9×10 ⁻³	19.2×10 ⁻³ ± 0.9×10 ⁻³
	3	1.4 ± 0.1	-27.8 ± 0.2	-25.9 ± 0.5	21.3×10 ⁻³ ± 1.5×10 ⁻³	19.8×10 ⁻³ ± 1.4×10 ⁻³
<i>Pinus sylvestris</i>	1	0.1 ± 0.1	-27.3 ± 0.2	-26.2 ± 0.2	11.9×10 ⁻³ ± 0.7×10 ⁻³	11.4×10 ⁻³ ± 0.7×10 ⁻³
	2	0.9 ± 0.0	-27.2 ± 0.1	-25.7 ± 0.3	16.4×10 ⁻³ ± 0.9×10 ⁻³	15.1×10 ⁻³ ± 0.7×10 ⁻³
	3	1.4 ± 0.1	-26.9 ± 0.2	-25.4 ± 0.6	20.6×10 ⁻³ ± 1.2×10 ⁻³	16.5×10 ⁻³ ± 0.8×10 ⁻³
<i>Picea abies</i>	1	0.1 ± 0.1	-26.9 ± 0.1	-26.2 ± 0.2	22.9×10 ⁻³ ± 1.0×10 ⁻³	17.9×10 ⁻³ ± 0.8×10 ⁻³
	2	0.9 ± 0.0	-27.2 ± 0.2	-25.4 ± 0.3	21.3×10 ⁻³ ± 1.3×10 ⁻³	18.4×10 ⁻³ ± 1.1×10 ⁻³
	3	1.4 ± 0.1	-27.2 ± 0.2	-25.3 ± 0.2	26.6×10 ⁻³ ± 1.5×10 ⁻³	21.9×10 ⁻³ ± 1.3×10 ⁻³